DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 12/6/2010 has been entered.

Amended claims 1, 10-12 and 16-22 are pending in the present application; and they are examined on the merits herein.

Response to Amendment

The rejection under 35 U.S.C. 112, first paragraph, for Enablement was withdrawn in light of Applicant's amendment.

New Matter

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Amended claims 1, 10-12 and 16-22 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the

application was filed, had possession of the claimed invention. ***This is a modified rejection.***

Amended independent claim 1 recites the limitation "**concentrating the fermentation harvest broth by centrifugation or micro filtration prior to and/or in conjunction with interruption of the further processing in step b)**". The amended clams encompass an embodiment in which **concentrating the fermentation harvest broth is conducted or carried out at a temperature of from about 4 ^{0}C to about 25 ^{0}C at a pH of from about 4 to about 10 and for a time ranging from at least about 1 hour to about 72 hours (to be in conjunction with interruption of the further processing in step b))**. The as-filed specification **does not have a written support for this broad limitation** in the amended method being claimed. While the as-filed specification has support for the step of concentrating the fermentation harvest broth **before OR after** the interrupting step (see page 10, second paragraph); and not in conjunction (at the same time or simultaneously or concurrently), particularly under the same recited conditions for the interrupting step b), as encompassed by the instant amended claims. In the amendment filed on 12/14/09 (page 5), Page 10, lines 13-14 of the as-filed specification state explicitly "The concentration of the fermentation harvest broth may be done before or after the interruption step, but preferably is done before". Applicants simply stated that support for the amendments may be found in the original claims and in the specification, without citing any specific page and/or line numbers. Original claims, including original claims 14-15 do not support the instant claims as

broadly written. Thus, there is **no written support** in the originally filed specification for the method as presently claimed.

Therefore, given the lack of sufficient guidance provided by the originally filed specification, it would appear that Applicants did not contemplate and/or had possession of the instant claimed invention at the time the application was filed.

Response to Arguments

Applicants' arguments with respect to the above rejection in the Amendment filed on 12/6/2010 (page 7) have been fully considered but they are respectfully not found persuasive.

Applicants argue that a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing even if every nuance of the claims is not explicitly described in the specification. Here, the concentration step would be understood by a person of ordinary skill to refer to embodiments in which the harvest broth is concentrated "before" and/or during the occurrence of the "interruption" step by reason of the explicit and unquestionable support at page 10 of the application. Additionally, the claims are open and therefore other steps may be carried out between the duration and occurrence of the fermentation and concentration and/or interruption steps; and the concentration step and/or other steps may in certain embodiments blend into or overlap with the interruption step. Accordingly, the instant specification met the Written description requirement; and the rejection should be withdrawn.

The as-filed specification does not teach or suggest that concentrating the fermentation harvest broth by centrifugation or microfiltration in conjunction (simultaneously) with the interrupting step b). Even assuming that the concentrating step is the interrupting step by itself (in conjunction), the as-filed specification also does not have a written support for the concentrating step to be conducted specifically under **the recited non-lethal conditions at a temperature of from about 4 ^{0}C to about 25 ^{0}C at a pH of from about 4 to about 10 and for a time ranging from at least about 1 hour to about 72 hours as encompassed by the instant claims.**

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Amended claims 1, 10-12 and 17-22 are rejected under 35 U.S.C. 102(b) as being anticipated by Bochner et al (US 4,680,262; IDS). ***This is a modified rejection.***

Bochner et al discloses a method for recovering periplasmic proteins, including heterologous eukaryotic proteins such as hGH, an interferon or a lymphokine, from transformed gram negative bacteria (see at least Summary; col. 4-6). In an exemplification, Bochner et al. teach the preparation of hGH from transformed *E. coli*, said method comprises culturing a transformant of *E. coli* W3110 tonA, phoA, phoT containing pAP-STII-hGH in 500 mL LB medium and O tetracycline at 37 ^{0}C for 8 hrs;

followed by seeding the 500 mL inoculum culture into the 10L fermenter containing phosphate-limiting medium at 37 ^{0}C and pH 7.5 for 36 hours; after which 1-butanol is added to the fermenter and steam is immediately injected into the fermenter jacket so that the temperature of the tank rises rapidly to 50 ^{0}C, and it is held at this temperature for 10 minutes (see example 8). Then, the fermenter is rapidly cooled below 20 ^{0}C and **the cellular contents of the fermenter are harvested by centrifugation. The cell paste is first frozen at –20 ^{0}C and then transferred to –80 ^{0}C until further processing is required** (col. 5, lines 4-51). In the exemplification, Bochner et al also disclose that **prior to extraction by mixing the cell paste with 10mM Tris-HCl, pH8.0, the frozen cell paste at -80 ^{0}C is thawed overnight at 4^{0}C** (col. 12, lines 39-43).

Please note that the step in which **the frozen cell paste at -80 ^{0}C is thawed overnight at 4 ^{0}C prior to extraction** is considered to be an interrupting step prior to extraction, and in this step the concentrated harvest broth is maintained at 4 ^{0}C for overnight which encompasses a time period of about 12 hours or more. It is also noted that Bochner et al teach specifically that **the cell paste typically contains residual quantities of the fermented culture medium; and that the pH of the culture medium is at pH 7.5** (col. 5, lines 37-42). **The conditions of 4 ^{0}C, pH 7.5 and overnight (about 12 hours or more) are non-lethal conditions as recited in the claims**. It is also noted that **due to the open language of the term "comprising", the claimed method may contain additional steps prior to the interrupting step b)**

such as and not limiting to concentrating the harvest broth or killing cells in the harvest broth.

Accordingly, the teachings of Bochner et al meet every limitation of the instant broad claims. Therefore, the reference anticipates the instant claims as written.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 12/14/2009 (pages 5-8) have been fully considered but they are respectfully not found persuasive.

Applicants argue basically that the Bochner's process requires application of lethal heat conditions and alkanol exposure to kill the cells after completion of fermaentation and prior to any concentration step. While killing cells does stop growth, it is not a step of "interrupting" as called for in claim 1. The interruption called for in the present case is in the sense of "suspending" fermentation of cells under specified, non-lethal conditions; and there is no suggestion whatsoever in the Bochner reference that any interruption of fermentation should occur before cell growth or fermentation is finished. Applicants further noted that Bochner et al also teach to kill the cells with heat and alkanol, after which the residue is concentrated, frozen, thawed, and then finally processed to extract a protein of interest. However, Applicants argue that Applicants claim a process in which fermentation of concentrated medium is substantially suspended after recombinant polypeptide has been secreted into the cell periplasm, and where the medium is maintained under non-lethal, quiescent conditions, after which

the desired polypeptide is harvested with an improved yield compared to conventional processes that complete fermentation and associated cell growth. Additionally, Applicants claim a process that takes place at temperatures above about 4^0C and without requiring any additional organic or other reagents that would kill cells.

First, please note that **due to the open language of the term "comprising", the claimed method may contain additional steps prior to the interrupting step b) such as and not limiting to concentrating the harvest broth or killing cells in the harvest broth**. Applicants indicated clearly in the amendment filed on 12/6/10 (page 7, second full paragraph) that **the claims are open and other steps can be conducted before and/or overlap with the interruption step**.

Second, the conditions of 4 ^{0}C, pH 7.5 and overnight (about 12 hours or more) are non-lethal conditions as recited in the claims.

Accordingly, the teachings of Bochner et al still meet all the limitations of the claims as broadly written.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bochner et al (US 4,680,262; IDS) in view of Wisniewski, R (US 6,337,205). ***This is a modified rejection.***

Bochner et al discloses a method for recovering periplasmic proteins, including heterologous eukaryotic proteins such as hGH, an interferon or a lymphokine, from transformed gram negative bacteria (see at least Summary; col. 4-6). In an exemplification, Bochner et al. teach the preparation of hGH from transformed *E. coli*, said method comprises culturing a transformant of *E. coli* W3110 tonA, phoA, phoT containing pAP-STII-hGH in 500 mL LB medium and O tetracycline at 37 ^{0}C for 8 hrs; followed by seeding the 500 mL inoculum culture into the 10L fermenter containing phosphate-limiting medium at 37 ^{0}C and pH 7.5 for 36 hours; after which 1-butanol is added to the fermenter and steam is immediately injected into the fermenter jacket so that the temperature of the tank rises rapidly to 50 ^{0}C, and it is held at this temperature for 10 minutes (see example 8). Then, the fermenter is rapidly cooled below 20 ^{0}C and **the cellular contents of the fermenter are harvested by centrifugation. The cell paste is first frozen at –20 ^{0}C and then transferred to –80 ^{0}C until further processing is required** (col. 5, lines 4-51). In the exemplification, Bochner et al also disclose that **prior to extraction by mixing the cell paste with 10mM Tris-HCl, pH8.0, the frozen cell paste at -80 ^{0}C is thawed overnight at 4^{0}C** (col. 12, lines 39-43). Please note that the step in which **the frozen cell paste at -80 ^{0}C is thawed overnight at 4 ^{0}C prior to extraction** is considered to be an interrupting step prior to extraction, and in this step the concentrated harvest broth is maintained at 4 ^{0}C for

overnight which encompasses a time period of about 12 hours or more. It is also noted that Bochner et al teach specifically that **the cell paste typically contains residual quantities of the fermented culture medium; and that the pH of the culture medium is at pH 7.5** (col. 5, lines 37-42). **The conditions of 4 ^{0}C, pH 7.5 and overnight (about 12 hours or more) are non-lethal conditions as recited in the claims**. It is also noted that **due to the open language of the term "comprising", the claimed method may contain additional steps prior to the interrupting step b) such as and not limiting to concentrating the harvest broth or killing cells in the harvest broth**.

Bochner et al do not teach explicitly that the cell paste is frozen in a fermenter.

At the effective filing date of the present application, Wisniewski already disclosed the use of **cryopreservation vials of various shapes and sizes for storing various products including proteins, DNA, RNA, biological cells such as bacteria, fungi, yeasts, mammalian cells at temperatures from about -1^{0}C to about -200^{0}C** (see at least Summary of the Invention, particularly col. 2, lines 61-67; Figures 2-3, 7-10; col. 12, lines 11-14; and col. 13, lines 33-49).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the method taught by Bochner et al by also freezing and storing the cell paste at –80 ^{0}C in anyone of the cryopreservation vials taught by Wisniewski as discussed above until further processing. The cryopreservation vials, including those in the shape of a tissue culture flask such as those shown in Figure 3C, Figure 7A and Figure 8B, can also be

used as a fermentation flask/vial, and therefore they are indistinguishable from a fermenter.

An ordinary skilled artisan would have been motivated to carry out the above modification because Wisniewski already disclosed the use of cryopreservation vials of various shapes and sizes for storing various products including proteins, DNA, RNA, biological cells such as bacteria, fungi, yeasts, mammalian cells at temperatures from about -1^{0}C to about -200^{0}C.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Bochner et al and Wisniewski, coupled with a high level of skill of an ordinary skilled artisan in the relevant art. Therefore, the modified method resulting from the combined teachings of Bochner et al and Wisniewski is indistinguishable from the method as broadly claimed.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 12/14/2009 (pages 9-10) have been fully considered but they are respectfully not found persuasive.

Applicants argue basically that the Wisniewski reference does not cure the deficiency of the Bochner reference, specifically the non-lethal interruption hold conditions recited in claim 1.

Please refer to the examiner's responses to Applicants' same arguments on the deficiencies of the Bochner et al reference above; particularly the examiner's interpretation of the instant broad claims.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

1. Pluckthun et al (US 7,033,798; Cited previously) teach that **an overnight incubation is a 20h- incubation period** (see at least col. 8, line 63; col. 11, line 7; col. 12, line 9).

2. Ruelle, J-L (US 6,613,335; Cited previously) teaches that **frozen cell paste can be thawed at room temperature which is about 25^{0}C or less, for 60 minutes prior to extraction by resuspending the thawed cell paste in phosphate buffer for homogenization** (see at least example 4, particularly col. 33, lines 21-40 and col. 34, lines 19-20)

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Woitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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/QUANG NGUYEN/

Primary Examiner, Art Unit 1633